

AN INVESTIGATION OF THE POSSIBLE ROLE OF BITING MIDGES (DIPTERA, CERATOPOGONIDAE) IN THE TRANSMISSION OF ARTHROPOD-BORNE VIRUS DISEASES AT TOWNSVILLE.

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(One Text-figure.)

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Synopsis.

At Townsville, North Queensland, in November, 1955, the host preferences of local species of biting midges were investigated. *Culicoides austropalpalis*, *C. mackayensis* and *C. magnesianus* were shown to feed on bird; *C. molestus* on bird, flying fox and man impartially; and *C. ornatus* together with *C. subimmaculatus* on flying fox and man with a distinct possibility of bird feeding as well. The three species *C. molestus*, *C. ornatus* and *C. subimmaculatus* must be considered potential vectors of disease between flying fox, bird and man.

INTRODUCTION.

The City of Townsville, North Queensland, has long been known as a centre of periodic outbreaks of dengue fever. The possibility of other viruses being involved has been discussed by Rowan and O'Connor (1957) and Rowan (1957). It has also been suggested that flying foxes (*Pteropus* spp.) and certain migratory birds might be implicated as wild reservoirs for dengue or other related viruses, and evidence suggestive of this is recorded by O'Connor *et alii* (1955), Rowan and O'Connor (1957) and Rowan (1957).

If dengue or other related viruses are found in flying foxes or wild birds, then vectors for these must be sought other than the urban and domestic vector, *Aedes aegypti*, responsible for man to man transmission in human epidemics. The investigation of other possible vectors has been undertaken by Mackerras *et alii* (1955) and on several occasions by members of the staff of the Department of Entomology, School of Public Health and Tropical Medicine, University of Sydney. In the opinion of this Department, based on some years of investigation of the blood feeding habits of blood-sucking flies, concentration solely on mosquitoes as possible vectors would be unwise. In view of the typically coastal incidence of dengue fever outbreaks and sporadically accumulated evidence of the feeding on birds by biting midges, it was thought that any investigation of possible vectors should include a study of these insects.

Because of their small size, cryptic behaviour, and possibly because of a general belief that their life span was very brief, biting midges have received almost no attention as possible disease vectors as compared with the more conspicuous mosquitoes. A review of their known role as vectors of disease appeared in Australian literature in Lee (1948). Since that time biting midges of the genus *Culicoides* have been shown to be vectors of a virus disease, blue-tongue of sheep (Du Toit, 1944), intermediate hosts of *Haemoproteus nettionis* of ducks (Fallis and Wood, 1957), as well as the cause of an allergic dermatitis in horses (Riek, 1954), all previous records having been as intermediate hosts of various nematode parasites of man or cattle.

Feeding by *Culicoides* on birds has been proven occasionally in Australia (unpublished information), but perhaps the earliest pertinent record is that of Jellison and Philip (1933), who found abundant *Culicoides* in birds' nests built close to a stream, and of these many were engorged with blood.

Hence, in the work undertaken by the School of Public Health and Tropical Medicine, evidence of blood-feeding on birds and flying foxes was sought amongst both

mosquitoes and biting midges in selected habitats in the vicinity of Townsville. The results of the mosquito studies have been reported by O'Gower (1960).

The task of proving blood-feeding by biting midges on hosts other than man is not an easy one. Direct observation of diurnal feeding is possible only on quiet and co-operative domestic animals; with nocturnal feeders and wild bird or animal hosts only precipitin testing of the stomach contents of engorged specimens is likely to yield positive data. Even this technique is not as flexible as it is with mosquitoes, since the limited amount of blood taken precludes a wide range of tests and also makes it necessary to smear such specimens within a few hours of actual feeding. In order to effect the capture of significant material, not only must special techniques be used, but the investigator must have an intimate knowledge of the microhabitats worthy of exploitation. Hence in the following account of the Townsville work some stress is laid on both the techniques employed and the habitats investigated.

The study was undertaken in November, 1955, this being the end of the dry season at Townsville. Hence the species caught were predominantly associated with salt-water habitats and little light is thrown on the potentialities of those associated with fresh or brackish water. Observations during the wet season would be required in order to gain a fully representative coverage of the populations and host preferences of all species known to occur in the area.

TECHNIQUE.

Biting midges were taken by electric suction light trap (modified Du Toit type), acetylene light trap, tent trap, sweep net, and by aspirator. From time to time the light traps were baited with flying foxes in cages, or set in trees visited by flying foxes, or placed in the vicinity of domestic poultry. The tent trap was always baited with flying fox. Unfortunately no wild birds were available as trap bait. On a number of occasions the electric light trap was run throughout the night and cleared every three hours so that freshly fed specimens could be taken out and some idea of preferred feeding times obtained.

Catches were killed with chloroform vapour and sorted forthwith (light traps catch a vast variety of insects other than mosquitoes and biting midges). Engorged specimens were pinned and the blood smeared onto filter paper for source identification by the precipitin technique (Lee *et al.*, 1954). These smears were stored over silica gel in a refrigerator pending transfer to Sydney where the actual precipitin tests were performed by K. J. Clinton at the School of Public Health and Tropical Medicine. The pinned specimens were identified as far as possible while fresh, checked when dry, and further checks made by clearing and mounting any doubtful specimens. Other specimens from the traps were preserved in 70% alcohol, or on pins for later identification.

RESULTS (*Relating to Habitats*).

Of more than 60 collections, engorged material was taken in 24. These 24, yielding significant information, came from six localities which are discussed below in order of recession from the mouth of the Ross R. (see Map, Text-fig. 1).

1. *Townsville Regional Electricity Board Powerhouse (T.R.E.B.).*

The mangrove swamp near the outfall of the condenser cooling water. Of a total of 13 collections, engorged material was taken in 6 and the hosts identified in 5 of these.

2. *Boundary Road.*

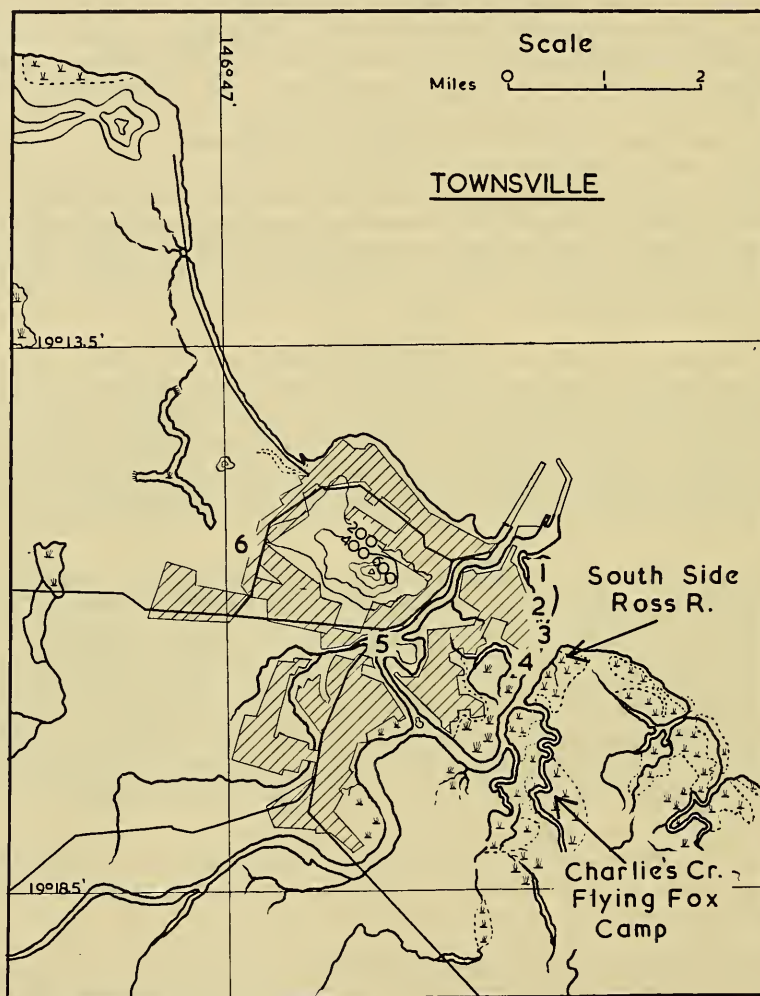
Some 400 yards upstream from 1, an unbaited electric suction trap was used for one night only set among mangroves 200 feet from high water mark towards the stream. In 4 collections only 2 engorged specimens of *Culicoides molestus* were taken for which no host identification could be made.

3. *Ross River Bank.*

At a house a little upstream from 2, on the shore above high water mark. Seven collections were made, all of which contained engorged material and in all cases the host was identified. The electric suction trap was used in a mango tree over flying-fox bait and the acetylene trap was set among poultry.

4. Bundy Creek.

A location further up the river where the channel is closer to the bank and there is relatively less mangrove growth on the town side of the river. The unbaited electric suction trap was set in a mango tree at 20 feet above ground level for one night. Four collections were made, each yielding engorged *Culicoides molestus*, and the hosts were identified. One collection aspirated from human bait yielded *Culicoides ornatus*.



Text-fig. 1. Map of Townsville with collection sites referred to in text.

5. Lowth's Bridge.

An area of mangrove swamp and salt pan intersected by deep tidal creeks adjacent to the railway yards. Of six collections, three yielded engorged material from which identification of the host also resulted. A sweep net, aspirator and flying-fox baited tent trap were also used here. The baited tent trap proved most effective when used with closed end towards maximum light, in this case sunrise.

6. Belgian Gardens.

In this suburb on the northern side of the city the electric suction trap was set unbaited in a mango tree 12 feet above ground. The tree was visited by flying foxes,

but in ten collections only one engorged specimen of *Culicoides austropalpalis* was taken. This gave a positive reaction for flying fox (but see under that species below).

Further small collections were made on the south side of Ross River. *Styloconops* sp. was taken among shoreline dunes near the mouth of the river. This species attacked man in numbers in the late afternoon. At a flying fox camp in the mangrove swamp along the upper part of Charlie's Creek *Culicoides ornatus* was taken attacking flying fox on the ground, and man, at about noon.

RESULTS (Relating to Species of Biting Midge).

Species Associated with a Freshwater Habitat.

1. *Culicoides austropalpalis* Lee & Reye.

This was present in all heavily collected localities and was the dominant species at Belgian Gardens and Ross River bank. As this species is believed to breed in freshwater pond margins its activity within and near mangrove foreshores was surprising. Since the proportion of males caught was obviously less in these areas, the possibility of a reasonably wide flight range is suggested. Under favourable conditions this species seemed to be active throughout the night, although with a fall in temperature the numbers fell off after midnight. Host identification showed a marked preference for bird, presumably fowl, for when poultry baited traps were used, up to 45% of the females captured were engorged. The finding of flying fox blood in a single specimen taken in an unbaited trap at Belgian Gardens must be considered atypical in the absence of further confirmation of feeding on this host.

2. *Culicoides dycei* Lee & Reye.

This was relatively rare and only females were taken. None were engorged, but at Belgian Gardens most were taken with the flying fox baited trap (midnight to 0300 hours) and one specimen was taken coming to man at Lowth's Bridge.

3. *Culicoides magnimaculatus* Lee & Reye.

Taken only at Belgian Gardens, this species was not common, nor was engorged material collected, although the best catches were with flying fox baited traps. *C. magnimaculatus* is known to be a diurnally active mammal-biting species in other localities.

4. *Culicoides marksii* Lee & Reye.

This was less common than *C. magnimaculatus*, but again the largest catch was with flying fox baited trap. No engorged material was taken of this known mammal-biting species. Apart from single specimens from Lowth's Bridge and Bundy Creek it was also confined to Belgian Gardens.

Species Associated with a Saltwater Habitat.

5. *Culicoides mackayensis* Lee & Reye.

Although uncommon, this species seemed to favour the mangrove flat area well inside the mouth of the Ross River where its activity was spread evenly throughout the night. Small numbers (including males) taken at Belgian Gardens probably came from the creek north of Kissing Point. Engorged material was taken only from the poultry baited trap at Ross River bank and this showed the host to be bird.

6. *Culicoides magnesianus* Lee & Reye.

Although taken in all localities, this species dominated the electric suction trap catches on the south bank of the Ross River near its mouth. Maximum nocturnal activity appeared to be between 2100 and 0300 hours. The few engorged specimens taken (one from a trap baited with flying fox) had fed on birds.

7. *Culicoides molestus* (Skuse).

This species was widely distributed in the lower part of Ross River, its proportion in catches increasing up the river to Bundy Creek where it became the dominant species. Its nocturnal activity seemed to be at a peak towards midnight and least before dawn. In this series the proportions of engorged to total females in collections of this species

were of the same order whether taken by trap baited with flying fox, or with poultry, or taken attacking man. Despite the proximity of all three baits at times, the engorged specimens had invariably fed on the bait of the particular trap. This is the only species so far proven to feed on man, flying fox and bird. *C. molestus* did not seem to range far from mangroves and the few taken at any distance from this habitat were not engorged.

8. *Culicoides ornatus* Taylor.

Although widely distributed, this species was found most abundantly in mangrove swamps penetrated by narrow creeks. This species presents taxonomic difficulties which will be discussed elsewhere, and may well resolve into a complex of species with diagnostic characters only in the male sex. For the time being the name *ornatus* will be applied to all females in this complex. Baited light traps were not used in the area of maximum abundance (Lowth's Bridge) and the only index of activity is attack on man. This seems to be at its peak just after sundown and just before sunrise. However, they were taken feeding on flying fox and man at noon in the flying fox camp on Charlie's Creek in the still shade of the mangrove swamp. Some idea of the attack rate is given from the results of a flying fox baited tent trap at ground level between 0500 and 0530 hours at Lowth's Bridge. Using a mouth operated aspirator, 380 females were taken, and of these 236 had fed, and the method of capture proved inadequate to cope with the numbers present. Some engorged specimens were taken in a poultry baited light trap, but, as the host was not identified, bird feeding by this species remains an unproven possibility.

9. *Culicoides subimmaculatus* Lee & Reye.

With a wide distribution within the river mouth this species appeared to dominate where the foreshore consisted of mangroves or sandy mud flats, e.g., T.R.E.B. to Boundary Road. Like *C. molestus*, it was not taken at any distance from the river. In traps the peak activity seemed to be about midnight. Like *C. ornatus*, it readily feeds on man and flying fox, and though no proof was obtained that it feeds on bird, the high proportion of fed material in bird-baited traps is suggestive that it does so.

10. *Culicoides marmoratus* (Skuse).

This intertidal zone species was less numerous than expected for this area, only 12 males and 35 females being taken in all, spread over 11 light trap collections. Most were taken in unbaited light traps at Bundy Creek and Belgian Gardens with a few at T.R.E.B. Traps baited with fowl and flying fox at Ross River bank produced single specimens only. No engorged material was taken nor any preferred time of activity shown. From other areas this species is known as a mammal biter often ranging widely from its habitat, being captured inland with the salt-marsh mosquito, *Aedes vigilax*.

11. *Styloconops* sp.

This was encountered in large numbers attacking man in the late afternoon among shore-line dunes on the southern side of Ross River. As far as is known, this is a diurnal and/or crepuscular species associated with certain types of sandy foreshore. What its natural hosts are in this terrain is in considerable doubt, and the presence of one specimen in a flying fox baited trap at T.R.E.B. is of doubtful significance.

Table 1 summarized the findings relative to hosts and habits.

DISCUSSION.

Within the limits of season and technique it appears that the three common species of *Culicoides* (*molestus*, *ornatus*, *subimmaculatus*) associated with mangroves may be considered as potential vectors of disease among the three hosts used in this survey (man, flying fox, bird). At present *C. molestus* seems the most likely as it attacks all three equally; *C. ornatus* and *C. subimmaculatus* readily attack man and flying fox and there is a distinct possibility that they may also attack birds. All three are possibly active in these mangrove swamps when flying foxes are in camp and birds are roosting.

Since this field work was done other surveys (unpublished) have indicated that the population at least of *C. subimmaculatus* and *C. ornatus*, and probably that of *C. molestus* also, waxes and wanes in relation to the lunar tide cycle. A prior knowledge of this phenomenon could have led to more appropriate timing of trapping at Townsville and to negative results being seen in a different perspective. It is expected that the *Styloconops* sp. taken will also have a similar relationship.

The remaining species associated with the intertidal zone (*C. mackayensis* and *C. magnesianus*) remain as bird-biting species, although there are occasional records from other localities of solitary specimens of *C. magnesianus* apparently attracted to man.

TABLE 1.

SPECIES OF BITING MIDGE.	HOST.			BITING ACTIVITY.
	Flying Fox.	Bird.	Man.	
<i>Culicoides austropalpalis</i> ..	(1) + ¹	(36) + D, B	—	Nocturnal, 1800-0600
<i>Culicoides dycei</i>	? ²	—	? ³	Nocturnal, 0-0300
<i>Culicoides magnimaculatus</i>	? ²	—	—	Diurnal and crepuscular
<i>Culicoides marksii</i>	? ²	—	—	Probably crepuscular.
<i>Culicoides mackayensis</i> ..	—	(1) + D	—	Nocturnal, 1800-0600
<i>Culicoides magnesianus</i> ..	—	(2) + B	—	Nocturnal, 2100-0300
<i>Culicoides marmoratus</i> ..	—			
<i>Culicoides molestus</i>	(7) +	(23) + D, B	+	Nocturnal, 1800-0600 and crepuscular
<i>Culicoides ornatus</i>	(84) +	? ⁴	(6) +	Crepuscular and diurnal
<i>Culicoides subimmaculatus</i>	(44) +	? ⁴	(1) +	Nocturnal and crepuscular
<i>Styloconops</i> sp.	? ⁵	—	+	Diurnal.

Notes.—Figures in brackets indicate numbers of positive precipitin reactions obtained; + indicates feeding on host demonstrated by observation or precipitin tests; D indicates feeding on domestic poultry and B indicates that the avian blood source was possibly other than a domestic one.

¹ Feeds predominantly on birds, this single positive is somewhat dubious.

² Females taken in baited light trap.

³ One specimen coming to man.

⁴ Fed specimens in poultry-baited light trap but host not identified.

⁵ Unfed specimen in flying fox baited light trap, doubtful as usually diurnal.

With the exception of the bird-biting *C. austropalpalis*, the species associated with fresh-water were present in such small numbers that little can be said of them from this survey. *C. magnimaculatus*, *C. marksii* and *C. dycei* are known to bite mammals elsewhere, but knowledge of other possible hosts or preferences among mammals must await further study; their presence in the seasonal conditions encountered suggests that they may be important after sufficient rain has fallen to expand the larval habitats.

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References.

- DU TOIT, R. M., 1944.—The Transmission of Blue Tongue and Horse Sickness by *Culicoides*. *Onderstepoort J. Vet. Sci. & Animal Ind.*, 19: 7-16.
- FALLIS, A. M., and WOOD, D. M., 1957.—Biting Midges (Diptera, Ceratopogonidae) as Intermediate Hosts for *Haemoproteus* of Ducks. *Canad. J. Zool.*, 35: 425-435.

- JELLISOW, W. L., and PHILIP, C. B., 1933.—Fauna of Nests of the Magpie and Crow in Western Montana. *Canad. Entom.*, 65: 26-31.
- LEE, D. J., 1948.—Australian Ceratopogonidae (Diptera, Nematocera). Part I. Relation to Disease Biology, General Characters and Generic Classification of the Family, with a note on the genus *Ceratopogon*. *Proc. Linn. Soc. N.S.W.*, 72: 313-331.
- LEE, D. J., CLINTON, K. J., and O'GOWER, A. K., 1954.—The Blood Sources of some Australian Mosquitoes. *Aust. J. biol. Sci.*, 7: 282-301.
- MACKERRAS, I. M., MARKS, E. N., and DOMROW, R., 1955.—*Ann. Rept. Q'ld. Inst. Med. Res.*: 8.
- O'CONNOR, J. L., ROWAN, L. C., and LAWRENCE, J. J., 1955.—Relationship between the Flying Fox (Genus *Pteropus*) and Arthropod-borne Fevers of North Queensland. *Nature*, 176: 472.
- O'GOWER, A. K., 1960.—Townsville Culicines as possible vectors of Dengue and Allied Viruses among local Feral Fauna. *Aust. J. expt. Biol. & Med. Sci.*, 38: 1-9.
- RIEK, R. F., 1954.—Studies on Allergic Dermatitis (Queensland Itch) of the Horse: The Aetiology of the Disease. *Aust. J. Agric. Res.*, 5: 109-129.
- ROWAN, L. C., 1957.—Recent Work on Dengue Fever. *Med. J. Aust.*, 2: 531-533.
- ROWAN, L. C., and O'CONNOR, J. L., 1957.—Relationship between some Coastal Fauna and Arthropod-borne Fevers of North Queensland. *Nature*, 179: 786-787.